Association of High-density Lipoprotein Cholesterol With Improvement of Endothelial Dysfunction Recovery in Renovascular Disease

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Introduction. This study was aimed to assess the ratio of total cholesterol (TC) to high-density density lipoprotein cholesterol (HDLC) and plasma nitrate levels in patients with ischemic nephropathy receiving statins and niacin extended release (NER).
Materials and Methods. Kidney disease patients with a history of at least 5 year of diabetes mellitus or 10 year of hypertension were screened by renal artery Doppler ultrasonography. Participants were randomly assigned into two groups to receive atorvastatin, 20 mg/d, with and without NER, 500 mg/d, for 16 weeks. Serum levels of lipid profile, creatinine, and nitrate were compared before and after the study.
Results. Fifty-four patients received the statin and 51 received statin-NER combination. Both statin and statin-NER groups demonstrated significant decreases in plasma levels of TC and low-density lipoprotein cholesterol. Triglyceride and very low-density lipoprotein cholesterol were significantly lowered only with statin-NER combination. The increase in HDLC level was found in both groups, but significant only with statin-NER combination therapy ($P < .001$). Atorvastatin combined with NER reduced TC/HDLC ratio almost double as compared with that of atorvastatin alone (102% and 36.6% reduction, respectively). A similar pattern was observed for nitrate levels (33% and 65%, respectively).
Conclusions. These findings indicated that a reduction in TC/HDLC ratio improves endothelial function in renovascular disease and use of NER in combination with atorvastatin may provide better outcomes. This could be helpful in attenuating further vascular damage and associated systemic complications.

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INTRODUCTION

Renovascular disease or ischemic kidney disease is abnormality of kidney perfusion resulting in significant glomerular filtrate reduction and nonresponsive hypertension to angiotensin-converting enzyme inhibitors that progressively damages functional parenchyma, leading to end-stage renal disease.[1] It was attributed as an independent cause of renal insufficiency in 1980s.[2] It may present alone or secondary to coronary artery disease, shares clinical findings and risk factors similar to ischemic heart disease such as advance age (> 50 years old), male sex, smoking, hypertension, diabetes mellitus, obesity, sedative lifestyle, family history of artery disease, and hypercholesterolemia.[3] The basic pathophysiology includes plaque formation in the renal artery following dyslipidemia, producing occlusion,

thrombosis, embolism, or atherosclerosis, exposing kidneys to ischemia and consequently collapsed glomeruli, tubular atrophy, and interstitial fibrosis.[4-6]

Atherosclerotic artery disease plays a crucial role in endothelial damage. Endothelial dysfunction is one of the early events of atherosclerosis, even before the evidence of plaques formation appear on diagnostic tests such as angiography or ultrasonography. This impaired endothelial function indicates damaging effects on bioactivity of nitric oxide (NO) and accelerated synthesis of reactive oxygen species.[7] Nitric oxide is considered as an atherosclerosis-protecting agent through its actions including monocyte migration inhibition, smooth muscle cell proliferation deceleration, and opposing of platelet aggregation.[5,8]

Nitrites and nitrates are the stable oxidation metabolites of endothelium-derived NO and are used to assess the total amount of NO in plasma.[9] Elevated NO oxidative products exhibiting NO deficiency have been reported in atherosclerotic vascular derangements and in chronic kidney disease.[5,9] On the other side, increased plasma total cholesterol (TC) is an established independent risk factor of atherosclerosis,[10] which also directly affects endothelial function,[11,12] either by slowing down formation and secretion of endothelium-derived relaxing factors or by superoxide radicals-dependent NO inactivation.[13] Similarly, high-density lipoprotein cholesterol (HDL) anti-atherosclerotic actions are well recognized. The mechanisms through which HDLC produces these effects include increased activity of reverse cholesterol transport, low-density lipoprotein cholesterol (LDL) oxidation inhibition, anti-inflammatory potential, platelet aggregation, cellular adhesion molecules expression, apoptosis of endothelial cell, endothelial function preservation, and re-endovascularization.[14-16]

Substantial studies are suggestive of reduced TC/HDLC ratio lowers the risk of atherosclerosis-related morbidity and mortality.[17,18] Myoishi and colleagues[19] reported an improvement in endothelium-dependent vasodilation following cholesterol-lowering therapy in normocholesterolemic individuals; however, the impact of TC/HDLC ratio changes on endothelium function in renovascular disease is less investigated. The present study was aimed to assess the effect of TC/HDLC ratio on endothelial function by measuring the plasma nitrate levels in patients with renovascular disease. Simultaneously, the effects of cholesterol-lowering statins alone and in combination with HDLC niacin extended release (NER) on TC/HDLC ratio and endothelial function were also evaluated in patients with renovascular disease.

MATEREIALS AND METHODS

Study Design and Participants

The study was an experimental randomized trial conducted in collaboration with the Department of Nephrology, Jinnah Postgraduate Medical Centre. Primarily patients visiting the outpatient unit with renal impairment and a history of at least 5 years of diabetes mellitus or 10 years of hypertension were suspected to have renal ischemic disease. A renal artery Doppler ultrasonography was used as a diagnostic tool, while presence or absence of ischemia was an independent decision of nephrology consultant. Based on the clinical presentation and observation from the Doppler ultrasonography, 135 patients were identified asked to give written consent to be a part of a follow-up study. Principles outlined in declaration of Helsinki were followed throughout the study.

Patients younger than 18 years or older than 70 years; those who had undergone a previous revascularization procedure, kidney transplantation, steroid therapy, or statin therapy; those with coexistence of chronic inflammatory diseases; and those who failed to follow the 4-week statins washout period were excluded. Patients referred from other medical units who had severe renal loss and immediately in need for dialysis were not included.

Study Protocol

Fifteen of the selected patients refused to participate and the remaining were randomly assigned in a 1:1 ratio to receive either atorvastatin, 20 mg/d, or NER, 500 mg/d, in combination with the same dose of atorvastatin for 16 consecutive weeks. At the start and end of the study, blood samples were collected from the antecubital vein, following a 12-hour fasting, in heparinized tubes and immediately centrifuged at 3000 rmp for 5 minutes. The separated plasma was kept frozen at -80°C in small 0.5-mL cuvettes till biochemical evaluation of lipid profile, creatinine, and nitrate.

Biochemical Evaluation

Total cholesterol, HDLC, triglyceride, and creatinine were measured in plasma using research grade spectrophotometric Randox kits, while LDLC and very low-density lipoprotein cholesterol (VLDLC) were calculated using the Friedwal formula. Plasma nitrate was measured by ion-selective electrodes using an ion meter 3345, according to the manufacturers' manual operating procedure. Briefly, ion meter was calibrated from a series of standard concentration (10 ppm to 100 ppm) made from stock (1000 ppm) by adding 2 M of $(NH_4)_2SO_4$ ionic strength adjusting buffer. Before use, the electrode was rinsed with deionized water and blotted dry. The apparatus was calibrated from low to higher concentration by placing electrode in standard and observed a stable reading. For sample preparation, 1.7 mL of deionized water, 0.2 ml of ionic strength adjusting buffer, and 0.1 mL of plasma were taken in a clean glass tube and mixed well, the electrode was placed, and stable reading was recorded in mg/L.

Statistical Analysis

Continuous variables were reported as mean ± standard deviation and were analyzed using the SPSS software (Statistical Package for the Social Sciences, version 17.0, SPSS Inc, Chicago, Ill, USA) for comparisons of the mean values. *P* values less than .05 were considered significant.

RESULTS

A total of 120 patients with ischemic nephropathy were randomized into two groups of atorvastatin and Atorvastatin-NER combination therapy (n = 60, each). Six patients dropped out from the first group because they failed to take medicine or did not report back for follow-up. Five patients left from the second group with the same reasons and 4 patients discontinued combination therapy because of poor tolerance and indigestion problems. Overall 54 patients taking statin and 51 with statin-NER combination completed the protocol successfully for 4 months.

Most of the patients were men, and hypertension and diabetes mellitus were the two most prevailed etiological factors. Anemia was also a common finding. Chewable tobacco was the most existing acquired risk factor predisposing to atherosclerotic changes (Table 1). There was no significant difference in the baseline lipid profile, blood glucose level, kidney function measurements, and plasma nitrate between the two patient groups (Table 2).

Both statin and statin-NER groups demonstrated decreases in plasma levels of TC ($P < .001$ and $P < .001$), LDLC ($P < .01$ and $P < .01$), triglyceride ($P > .05$ and $P < .001$), and VLDLC ($P < .05$ and $P < .01$), respectively (Table 3). In the case of TC and LDLC, the reduction was significant while the levels of triglyceride and VLDLC were significantly lowered only in group treated with statin-NER combination. The increase in HDLC level was found in both groups, but significant only with statin-NER combination therapy ($P < .001$). In the

Table 1. Basic Characteristics of the Studied Patient Groups with Renovascular Disease Treated With Atorvastatin Alone and Atorvastatin in Combination with Niacin Extended Release (NER)

Characteristics	Statin Group (n = 60)	Statin-NER Group (n = 60)
Mean age, y	48.6 ± 9.9	46.7 ± 11.2
Sex		
Male	39 (65.0)	42 (70.0)
Female	21 (35.0)	18 (30.0)
Mean body mass index, kg/m^2	25.0 ± 3.2	24.0 ± 3.8
Diabetes mellitus	33 (55.0)	38 (63.3)
Hypertension	51 (85.0)	52 (86.6)
Cardiovascular disease	12 (20.0)	14 (23.3)
Anemia	27 (45.0)	24 (40.0)
Currently smoking	18 (30.0)	16 (26.6)
Previously smoking	6 (10.0)	8 (13.3)
Alcohol drinking	1 (1.6)	1 (1.6)
Oral tobacco use	36 (60.0)	34 (56.6)

Table 2. Baseline Biochemical Profile of Renovascular Disease Patients Treated with Atorvastatin Alone and Atorvastatin in Combination with Niacin Extended Release (NER)*

Parameters	Statin Group (n = 60)	Statin-NER Group (n = 60)
Total cholesterol, mg/dL	180.7 ± 35.9	178.8 ± 38.3
Low-density lipoprotein, mg/dL	113.49 ± 34.8	116.8 ± 39.2
High-density lipoprotein, mg/dL	22.93 ± 4.7	23.1 ± 5.4
Triglyceride, mg/dL	223.4 ± 28.5	216.0 ± 34.8
Very Low-density lipoprotein, mg/dL	46.9 ± 12.8	44.3 ± 11.6
Glomerular filtration rate, mL/min	66.9 ± 8.7	71.2 ± 12.4
Creatinine, mg/dL	4.1 ± 0.9	3.92 ± 1.1
Blood urea nitrogen, mg/dL	51.1 ± 5.2	48.6 ± 7.3
Fasting blood glucose, mg/dL	121 ± 11	126 ± 8
Hemoglobin, g/dL	9.3 ± 2.1	8.9 ± 1.8
Nitrate, mg/dL	0.64 ± 0.10	0.66 ± 0.13

*All values are mean ± standard deviation.

Table 3. Lipid Profile and Plasma Nitrate Before and After Treatment With Atorvastatin Alone and Atorvastatin in Combination with Niacin Extended Release (NER)*

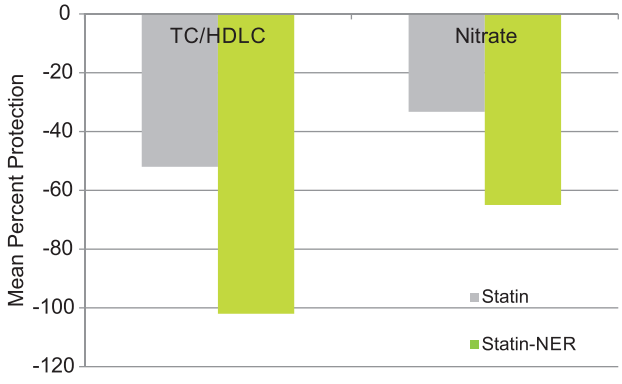
Parameter	Statin Group (n = 56)	Statin-NER Group (n = 51)
Total cholesterol, mg/dL		
Baseline	180.7 ± 35.9	178.7 ± 38.3
Follow-up	149.8 ± 3.8†	118.5 ± 4.6†
Triglyceride, mg/dL		
Baseline	223.8 ± 28.5	215.9 ± 34.8
Follow-up	195 ± 13.8	121.62 ± 25.4†
Low-density lipoprotein, mg/dL		
Baseline	113.5 ± 34.8	116.7 ± 39.2
Follow-up	96.3 ± 16.2‡	88.6 ± 15.7‡
High-density lipoprotein, mg/dL		
Baseline	22.9 ± 4.3	23.1 ± 5.4
Follow-up	30.1 ± 2.8	34.8 ± 3.1†
Very Low-density lipoprotein, mg/dL		
Baseline	46.9 ± 12.8	44.2 ± 11.6
Follow-up	37.3 ± 4.4	24.3.4 ± 3.5‡
Total cholesterol-high-density lipoprotein ratio		
Baseline	7.66 ± 0.9	7.33 ± 1.22
Follow-up	5.10 ± 1.30‡	3.60 ± 0.85†
Nitrate, mg/dL		
Baseline	0.64 ± 0.10	0.66 ± 0.13
Follow-up	0.48 ± 0.09‡	0.40 ± 0.11†

*All values are mean ± standard deviation.
†$P < .001$ compared to baseline
‡$P < .01$ compared to baseline

patients treated with statin-NER combination, this increment was more pronounced as compared to statin alone in terms of percentage of improvement from baseline (63.4% and 30.4%, respectively). Atorvastatin alone and in combination with NER significantly reduced TC/HDLC ratio ($P < .01$ and $P < .001$) and plasma nitrate ($P < .01$ and $P < .001$; Table 3); however, the mean protection provided by the latter treatment comparatively produced better outcomes. Atorvastatin combined with NER reduced TC/HDLC ratio almost double as compared with that of atorvastatin alone (102% and 36.6% reduction, respectively). A similar pattern was observed for nitrate levels (33% and 65%, respectively).

DISCUSSION

The present study examined in vivo effects of cholesterol-lowering atorvastatin on NO bioavailability measured in terms of its stable metabolite, plasma nitrate levels in comparison to HDLC-enhancing NER. The collected data showed that a reduction in cholesterol concentration improved the endothelial function and a decrease in TC/HDLC ratio was positively related with plasma nitrate level during ischemic changes (Table 3). Atorvastatin reduces cholesterol as a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor, but it has less effect on plasma HDLC; thus, changes in TC/HDLC ratio was comparatively less as compared to that by NER; overall change in TC/HDLC ratio was found to be higher in the patients treated with statin-NER combination (Figure).



Mean percent protection induced by atorvastatin alone and atorvastatin in combination with niacin extended release (NER) in terms of total cholesterol-high-density lipoprotein cholesterol (TC/HDLC) ratio and plasma nitrate in patients with renovascular disease. Negative sign indicates reduction in baseline value.

It is well recognized that hypercholesterolemia plays a key role in the development of atherosclerosis by provoking or contributing in many injurious pathways, one of which is suppressed bioavailability of NO that disrupts the endothelial function.[20] In the pathophysiology of atherosclerosis, altered oxidant-antioxidants balance also contributes by causing lipid peroxidation in the arterial macrophages and lipoproteins.[21] The comprehensive result of both mechanisms is a fall in endothelial NO and reduced effective blood vessel lumen.

Substantial data show that HDLC possesses beneficial effect in the retardation of atherosclerotic process by removing excessive cellular cholesterol to the liver by reverse cholesterol transport.[14,15] It also offers vascular function protection in a multifactorial fashion; increases NO bioavailability by activating endothelial NO synthase by Akt and MAP kinase enhancement and by inhibiting caspase 3 and 9; restrains cellular apoptosis in vascular endothelium; arrests the LDLC oxidation; and slows down inflammatory processes, anticoagulation, and vasodilation by kindling prostacyclin synthesis.[14,15] In low-HDLC status, as we saw in ischemic kidney disease patients, atherosclerosis progresses rapidly.

Harangi and colleagues[22] reported that treatment with atorvastatin in hyperlipidemic individuals reduced the oxidative stress while short-term betterment in the endothelial function by same drug has been described by Bleda and colleagues[23] in patients with peripheral artery disease, the two atherosclerosis retarding outcomes. A possible mechanism by which atorvastatin improves endothelial function may be its potential to reduce TC/HDLC ratio.[23] It means it is not the cholesterol reduction merely, but a decrease in this TC/HDLC ratio has a positive effect on endothelium, so HDLC-elevator combination with atorvastatin would produce more pronounce effect on endothelial function recovery, the same that we have demonstrated by adding NER to atorvastatin treatment.

Currently, niacin is the most effective available medical treatment to increase HDLC. Its HDLC-elevating potential is accompanied by a decrease in other atherogenic lipids, including LDLC, TC, triglyceride, and VLDLC.[24,25] It has been described that niacin mainly acts on adipose tissue where it attenuates the VLDLC synthesis that in turns limits the exchange of cholesterol from HDL to VLDLC, triglyceride from VLDLC to HDLC, cholesterol between HDLC and LDLC, all of which are mediated by cholesterol ester transfer protein.[24,26] The overall effect is dropped HDLC catabolism and low accumulation of cholesterol esters in LDLC particles. Many studies have shown that niacin further increase plasma HDLC level by directly inhibiting ApoA1-containing HDLC particles catabolism and their uptake.[27,28]

Sugiura and colleagues[29] previously reported inverse correlation between TC/HDLC ratio and endothelial function using flow-mediated dilation technique that is in agreement of our findings as we observed the same using plasma nitrate level, indirect evaluation of NO and endothelial function. A similar study that supports our results was done by Bleda and coworkers[30] who assessed the effect of TC/HDLC ratio in peripheral artery disease patient endothelial function by measuring plasma nitrite, another stable metabolite of NO using atorvastatin as a cholesterol lowering drug. They concluded that the observed relationship between TC/HDLC ratio and endothelial function is mainly because of HDLC increase, but they used a drug meant for cholesterol reduction, while we administered and compared an HDLC-elevating agent to see the effect of TC/HDLC ratio on endothelial function.

The study limitation encompasses not investigating the other nitrate-influencing factors including dietary nitrates, atmospheric NO inhalation, and formation in salivary glands. Although it is not possible to exclude these factors, our data is in accordance with the previously reported studies on statin. Moreover, dealing only with nitrates and not nitrites may be a limitation. This is a primary study and further investigation is needed in kidney disease patients.

CONCLUSIONS

The findings of this study showed that a reduction in TC/HDLC ratio improves endothelial function in renovascular disease and use of NER in combination with atorvastatin may provide better desired outcome. This could be helpful in attenuating further vascular damage and associated systemic complications.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Adamczak M, Wiecek A. Ischemic nephropathy - pathogenesis and treatment. Nefrologia. 2012;32:432-8.
2. Coen G, Manni M, Giannoni MF, et al. Ischemic nephropathy in an elderly nephrologic and hypertensive population. Am J Nephrol. 1998;18:221-7.
3. Garcia-Donaire JA, Alcazar JM. Ischemic nephropathy: detection and therapeutic intervention. Kidney Int Suppl. 2005;S131-S136.
4. Khatami MR. Ischemic nephropathy: more than a simple renal artery narrowing. Iran J Kidney Dis. 2013;7:82-100.
5. de Haro MJ, Martinez-Aguilar E, Florez A, Varela C, Bleda S, Acin F. Nitric oxide: link between endothelial dysfunction and inflammation in patients with peripheral arterial disease of the lower limbs. Interact Cardiovasc Thorac Surg. 2009;9:107-12.
6. Lerman L, Textor SC. Pathophysiology of ischemic nephropathy. Urol Clin North Am. 2001;28:793-803, ix.
7. Ignarro LJ, Cirino G, Casini A, Napoli C. Nitric oxide as a signaling molecule in the vascular system: an overview. J Cardiovasc Pharmacol. 1999;34:879-86.
8. De HJ, Acin F, Lopez-Quintana A, et al. Direct association between C-reactive protein serum levels and endothelial dysfunction in patients with claudication. Eur J Vasc Endovasc Surg. 2008;35:480-6.
9. Baylis C. Nitric oxide deficiency in chronic kidney disease. Am J Physiol Renal Physiol. 2008;294:F1-F9.
10. Zulli A, Hare DL, Buxton BF, Black MJ. High dietary methionine plus cholesterol exacerbates atherosclerosis formation in the left main coronary artery of rabbits. Atherosclerosis. 2004;176:83-9.
11. Mullen MJ, Kharbanda RK, Cross J, et al. Heterogenous nature of flow-mediated dilatation in human conduit arteries in vivo: relevance to endothelial dysfunction in hypercholesterolemia. Circ Res. 2001;88:145-51.
12. Zeiher AM, Drexler H, Saurbier B, Just H. Endothelium-mediated coronary blood flow modulation in humans. Effects of age, atherosclerosis, hypercholesterolemia, and hypertension. J Clin Invest. 1993;92:652-62.
13. Boger RH. Association of asymmetric dimethylarginine and endothelial dysfunction. Clin Chem Lab Med. 2003;41:1467-72.
14. Ansell BJ, Watson KE, Fogelman AM, Navab M, Fonarow GC. High-density lipoprotein function recent advances. J Am Coll Cardiol. 2005;46:1792-8.
15. Feig JE, Rong JX, Shamir R, et al. HDL promotes rapid atherosclerosis regression in mice and alters inflammatory properties of plaque monocyte-derived cells. Proc Natl Acad Sci U S A. 2011;108:7166-71.
16. Ozaki Y, Tanaka A, Komukai K, et al. High-density lipoprotein cholesterol level is associated with fibrous cap thickness in acute coronary syndrome. Circ J. 2013;77:2982-9.
17. Ray KK, Cannon CP, Cairns R, Morrow DA, Ridker PM, Braunwald E. Prognostic utility of apoB/AI, total cholesterol/HDL, non-HDL cholesterol, or hs-CRP as predictors of clinical risk in patients receiving statin therapy after acute coronary syndromes: results from PROVE IT-TIMI 22. Arterioscler Thromb Vasc Biol. 2009;29:424-30.
18. Real JT, Chaves FJ, Martinez-Uso I, Garcia-Garcia AB, Ascaso JF, Carmena R. Importance of HDL cholesterol levels and the total/ HDL cholesterol ratio as a risk factor for coronary heart disease in molecularly defined heterozygous familial hypercholesterolaemia. Eur Heart J. 2001;22:465-71.
19. Myoishi M, Hao H, Minamino T, et al. Increased endoplasmic reticulum stress in atherosclerotic plaques associated with acute coronary syndrome. Circulation. 2007;116:1226-33.
20. Rasmusen C, Cynober L, Couderc R. [Arginine and statins: relationship between the nitric oxide pathway and the atherosclerosis development]. Ann Biol Clin (Paris). 2005;63:443-55.
21. Rosenblat M, Hayek T, Hussein K, Aviram M. Decreased macrophage paraoxonase 2 expression in patients with hypercholesterolemia is the result of their increased cellular cholesterol content: effect of atorvastatin therapy. Arterioscler Thromb Vasc Biol. 2004;24:175-80.
22. Harangi M, Seres I, Varga Z, et al. Atorvastatin effect on high-density lipoprotein-associated paraoxonase activity and oxidative DNA damage. Eur J Clin Pharmacol. 2004;60:685-91.
23. Bleda S, de Haro J, Florez A, Varela C, Esparza L, Acin F. Long-term pleiotropic effect of statins upon nitric oxide and C-reactive protein levels in patients with peripheral arterial disease. Heart Asia. 2011;3:130-4.
24. Pang J, Chan DC, Hamilton SJ, Tenneti VS, Watts GF, Barrett PH. Effect of niacin on high-density lipoprotein apolipoprotein A-I kinetics in statin-treated patients with type 2 diabetes mellitus. Arterioscler Thromb Vasc Biol. 2014;34:427-32.
25. Mahboubi K, Witman-Jones T, Adamus JE, et al. Triglyceride modulation by acifran analogs: activity towards the niacin high and low affinity G protein-coupled receptors HM74A and HM74. Biochem Biophys Res Commun. 2006;340:482-90.
26. Gille A, Bodor ET, Ahmed K, Offermanns S. Nicotinic acid: pharmacological effects and mechanisms of action. Annu Rev Pharmacol Toxicol. 2008;48:79-106.
27. Lamon-Fava S, Diffenderfer MR, Barrett PH, et al. Extended-release niacin alters the metabolism of plasma apolipoprotein (Apo) A-I and ApoB-containing lipoproteins. Arterioscler Thromb Vasc Biol. 2008;28:1672-8.
28. Zhao SP, Yang J, Li J, Dong SZ, Wu ZH. Effect of niacin on LXRalpha and PPARgamma expression and HDL-induced cholesterol efflux in adipocytes of hypercholesterolemic rabbits. Int J Cardiol. 2008;124: 172-8.
29. Sugiura T, Dohi Y, Yamashita S, et al. Impact of lipid

profile and high blood pressure on endothelial damage. J Clin Lipidol. 2011;5:460-6.

30. Bleda S, De HJ, Varela C, Esparza L, Rodriguez J, Acin F. Improving Total-Cholesterol/HDL-Cholesterol Ratio Results in an Endothelial Dysfunction Recovery in Peripheral Artery Disease Patients. Cholesterol. 2012;2012:895326.

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